



TITLE OF THE INVENTION

Kits and Methods for Assessing Oxidative Stress

CROSS-REFERENCE TO RELATED APPLICATIONS

5 Not applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

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REFERENCE TO A MICROFICHE APPENDIX

Not applicable.

BACKGROUND OF THE INVENTION

15 **[0001]** Oxidation of the chemical components of foodstuffs provides energy that is used to build and maintain the body and to enable normal physiological function. Such oxidation involves a chain of chemical reactions including reactions in which transfer of electrons from one chemical compound to another are catalyzed. These reactions are catalyzed by enzymes, which serve to align and chemically activate one or more of, for example, reactants, cofactors,
20 metal atoms or ions, and water molecules. Despite the inherent specificity of enzyme-catalyzed reactions, side reactions inevitably occur.

[0002] Oxygen is a common and relatively chemically reactive component of biological systems. Diatomic oxygen is ordinarily relatively harmless to body systems, as is fully reduced oxygen (i.e., water). However, transfer of one or more electrons to oxygen (e.g., during
25 reduction of oxygen to water during oxidative phosphorylation or by way of a side reaction of another biochemical process) can result in formation of more reactive species of oxygen, such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals. These relatively reactive forms of oxygen can damage biochemical components of the body such as proteins, lipids, and DNA, destroying or inhibiting the normal function of the components.

[0003] The effects of biochemical damage inflicted by interaction of reactive forms of oxygen with body components can be manifested in a number of ways. DNA is the genetic material that carries the 'instructions' for making the components of a normal human body. Oxidative damage to DNA can result in mutations (i.e., changes in the 'instructions') that lead the body to make abnormal components. The abnormal components can have inhibited (or no) ability to perform their normal function, and this can be manifested as a disease or disorder. Likewise, oxidative damage to enzymes or lipid components of membranes can inhibit or ablate their normal function, and this too can be manifested as a disease or disorder. The degree to which a cell or tissue of a human body is subjected to damage caused by reactive forms of oxygen is sometimes designated 'oxidative stress.' The diseases and disorders associated with oxidative damage to body components are thus manifestations of oxidative stress. Aging is another manifestation of oxidative stress. Over time, damage caused by interaction of reactive forms of oxygen with body components degrades the structure and function of those components, leading to detectable changes in body structure and function.

[0004] If the human body were not able to detoxify reactive forms of oxygen and mitigate their effects on the body, then human life would be significantly shorter or even impossible. However, the human body comprises enzymes which are able to catalyze transformation of reactive forms of oxygen to less toxic species and other enzymes which are able to repair damage done to body components by reactive forms of oxygen.

[0005] Most, if not all, human genes occur in a variety of forms which differ in at least minor ways. Heterogeneity in human genes is believed to have arisen, in part, from minor, non-fatal mutations that have occurred in the genome over time. In some instances, differences between alternative forms of a gene are manifested as differences in the amino acid sequence of a protein encoded by the gene. Some amino acid sequence differences can alter the reactivity or substrate specificity of the protein. Differences between alternative forms of a gene can also affect the degree to which (if at all) the gene is expressed. However, many heterogeneities that occur in human genes appear not to be correlated with any particular phenotype. Known heterogeneities include, for example, single nucleotide polymorphisms (i.e., alternative forms of a gene having a difference at a single nucleotide residue). Other known polymorphic forms

include those in which the sequence of larger (e.g., 2-1000 residues) portions of a gene exhibits numerous sequence differences and those which differ by the presence or absence of portion of a gene.

[0006] Numerous disorders and physiological states have been correlated with occurrence of one or more alternative forms of a gene in the genome of a human who exhibits the disorder or physiological state. For example, Kimura et al. (2000, Am. J. Ophthalmol. 130:769-773) discloses an association between occurrence of a SNP of the manganese superoxide dismutase gene and a form of macular degeneration. Although associations between individual disorders and individual genetic polymorphisms are known, a need remains for a method of assessing the overall state of oxidative stress to which a human is subjected. The invention satisfies this need.

BRIEF SUMMARY OF THE INVENTION

[0007] The invention relates to a method of assessing relative susceptibility of a human to oxidative damage. The method comprises assessing occurrence in the human's genome of disorder-associated polymorphisms (e.g., single nucleotide polymorphisms; SNPs) in at least two (and preferably three, four, six, ten, fifteen, or twenty or more) genes selected from the group consisting of

- a) genes which encode an enzyme that catalyzes conversion of a toxic oxygen species to a less toxic oxygen species;
- b) genes which encode a protein that provides protection against oxidative stress;
- c) genes which encode a protein that induces production of a toxic oxygen species;
- d) genes which encode a protein that indirectly affects oxidative stress; and
- e) genes which encode a protein for which the level of expression of the protein is associated with oxidative stress.

[0008] Occurrence of any of the polymorphisms is an indication that the human is more susceptible to oxidative damage than a human whose genome does not comprise the polymorphism. Furthermore, occurrence of a plurality of the polymorphisms is an indication that the human is even more susceptible to oxidative damage than a human whose genome does

not comprise the polymorphisms. Preferably the genes are selected from the group consisting of a), b), c), and d), and more preferably they are selected from the group consisting of a), b), and c). In one embodiment, the method comprises assessing occurrence in the human's genome of disorder-associated polymorphisms in at least four genes selected from the group consisting of genes which encode an enzyme that catalyzes conversion of a toxic oxygen species to a less toxic oxygen species (e.g., genes which encode mitochondrial manganese superoxide dismutase, cytoplasmic copper/zinc superoxide dismutase, catalase, and glutathione peroxidase).

[0009] The method by which occurrence of an individual disorder-associated

polymorphism is assessed is not critical. For example, occurrence of the polymorphisms can be assessed using a method that includes contacting a nucleic acid derived from the human's genome with a first oligonucleotide. The first oligonucleotide can be one that anneals with higher stringency with the disorder-associated polymorphism than with a corresponding non-disorder-associated polymorphism. Annealing of the first oligonucleotide and the nucleic acid can be assessed, and such annealing is an indication that the human's genome comprises the disorder-associated polymorphism. Use of an oligonucleotide has the advantage that the oligonucleotide can be attached to a support using routine methods, and that a plurality of oligonucleotides can be attached to the same support, to allow simultaneous detection of multiple polymorphisms. If a second oligonucleotide which anneals with higher stringency with a non-disorder-associated polymorphism than with a corresponding disorder-associated polymorphism is used, then the allelic content of the human's genome can be determined. Detection of polymorphic sequences can be simplified by using labeled oligonucleotides, such as molecular beacon oligonucleotides.

[0010] Once the content of the human's genome for disorder-associated polymorphisms has been assessed, assessment of susceptibility to oxidative damage can further comprise calculating a susceptibility score for the human. A susceptibility score can be calculated by summing, for each of the selected genes in which a disorder-associated polymorphism occurs in the human's genome, the product of a constant and a correlation factor. The correlation factor can, alternatively, be a factor that represents the fraction of humans heterozygous for the

disorder-associated polymorphism who exhibit the corresponding disorder or a factor that represents the fraction of humans homozygous for the disorder-associated polymorphism who exhibit the corresponding disorder. The constant can be selected based on the known or surmised relevance of the gene with respect to oxidative damage. The susceptibility score represents the relative susceptibility of the human to oxidative damage.

[0011] In another aspect, the invention relates to a method of selecting a dose of an anti-oxidant composition (i.e., a composition comprising a compound that exhibits anti-oxidant properties, such as vitamin E or vitamin C, or a compound that can otherwise supplement the body's normal anti-oxidant mechanisms, such as alpha-lipoic acid and coenzyme Q) for administration to a human. This method comprises assessing occurrence in the human's genome of disorder-associated polymorphisms in at least one of the genes selected from the group consisting of a), b), c), d), and e), as indicated above. After assessing occurrence of the polymorphisms, a dose of the composition is selected. Occurrence of any of the polymorphisms is an indication that a greater dose of the composition should be administered to the human.

[0012] The invention also relates to a kit for assessing relative susceptibility of a human to oxidative damage. The kit comprises reagents for assessing occurrence in the human's genome of disorder-associated polymorphisms in at least one gene selected from the group consisting of a), b), c), d), and e), as indicated above. Examples of suitable reagents include oligonucleotides (e.g., molecular beacon oligonucleotides) that anneal with higher stringency with the disorder-associated polymorphisms than with corresponding non-disorder-associated polymorphisms and oligonucleotide primers that are complementary to the region adjacent a characteristic residue of the disorder-associated polymorphism. These primers are useful for amplifying at least the characteristic residue, thereby facilitating its detection. The kit can further comprise an instructional material which includes a numerical value representing the product of a constant and a correlation factor.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0013] The foregoing summary, as well as the following detailed description of preferred embodiments of the invention, will be better understood when read in conjunction with the appended drawings. The invention is not limited to the precise arrangements and instrumentalities shown.

[0014] Figures 1A and 1B are images which depict examples of results that can be obtained by analyzing occurrence of polymorphisms in several genes. The results shown in Figure 1A are derived from a hypothetical first human, and those shown in Figure 1B are derived from a hypothetical second human. Circles represent different polymorphisms of the gene indicated to the left of the row of circles. Filled circles indicate the presence of the polymorphism. Non-filled circles indicate the absence of the polymorphism. Numbers below each circle represent a correlation factor for the polymorphism and a disease or disorder.

DETAILED DESCRIPTION OF THE INVENTION

[0015] The invention relates to kits and methods for assessing the relative susceptibility of a human to oxidative damage by assessing occurrence in the human's genome of genetic polymorphisms that are associated with disorders. Crudely simplified, the methods involves determining whether one or more polymorphisms that have been associated (by the inventors or by others) with a disorder (e.g., a disease or pathological state) in humans occur in the genome of the human being tested. In some embodiments, the number of polymorphisms that occur in the human's genome are summed to yield a value; the higher the value is, the greater the susceptibility of the human to oxidative damage is assessed to be. In other embodiments, a weighting factor is assigned to each polymorphism tested, and the weighting factors of polymorphisms that occur in the human's genome are summed to yield a value that represents relative susceptibility to oxidative damage. The weighting factor can represent the product of a constant assigned to the gene in which the corresponding polymorphism occurs and a correlation factor that describes how informative occurrence of the polymorphism is for occurrence of the disorder with which it is associated. The invention includes a variety of alternative methods and kits for performing the methods, as described in greater detail herein.

Definitions

[0016] As used in this disclosure, the following terms have the meanings associated with them in this section.

[0017] A "polymorphism" in a gene is one of the alternative forms of a portion of the gene that are known to occur in the human population. For example, many genes are known to exhibit single nucleotide polymorphic forms, whereby the identity of a single nucleotide residue of the gene differs among the forms. Each of the polymorphic forms represents a single polymorphism, as the term is used herein. Other known polymorphic forms include alternative forms in which multiple consecutive or closely-spaced, non-consecutive nucleotide residues vary in sequence, forms which differ by the presence or absence of a single nucleotide residue or a small number of nucleotide residues, and forms which exhibit different mRNA splicing patterns.

[0018] A "single nucleotide polymorphism" ("SNP") is one of the alternative forms of a portion of a gene that vary only in the identity of a single nucleotide residue in that portion.

[0019] A "disorder-associated" polymorphism is an alternative form of a portion of a gene, wherein occurrence of the alternative form in the genome of a human has been correlated with exhibition by the human of a disease or a pathological state.

[0020] A "non-disorder-associated" polymorphism is an alternative form of a portion of a gene for which no significant correlation has been made between occurrence of the alternative form in the genome and a disease or a pathological state. Non-disorder-associated polymorphisms are sometimes designated "neutral" polymorphisms in the art.

[0021] A disorder-associated polymorphism and a non-disease-associated polymorphism "correspond" with one another if the two polymorphisms are two alternative forms of the same portion of the gene. By way of example, if the identity of residue 100 of a gene is adenine in a disorder-associated polymorphism of the gene and cytosine in a non-disorder-associated polymorphism of the gene, then the two polymorphisms correspond with one another. It is understood that there may be three or more corresponding polymorphisms when there are more than two alternative forms of the same portion of the gene.

[0022] A "characteristic residue" of a polymorphism is a nucleotide residue, the identity of which is known to vary among the alternative forms corresponding to the polymorphism.

[0023] "Toxic oxygen species" include, in approximate order of reactivity, hydroxyl radicals, superoxide radicals, nitric oxide, peroxy nitrite (ONOO⁻; the product of a reaction between nitric oxide and superoxide radical), and hydrogen peroxide. Ordinary diatomic oxygen is not a toxic oxygen species, as the term is used herein.

[0024] "Oxidative damage" refers to chemical reaction of a normal cellular component (e.g., DNA, a protein, or a lipid) with a toxic oxygen species, whereby at least one normal function of the component is inhibited or eliminated. The terms "oxidative damage" and "oxidative stress" are used interchangeably herein.

[0025] A "molecular beacon oligonucleotide" is a single-stranded oligonucleotides having a fluorescent label (e.g., rhodamine, FAM, TET, VIC, JOE, or HEX) attached to the 5'-end thereof and a fluorescence quencher (e.g., TAMRA or DABCYL) attached to the 3'-end thereof (or vice versa), as described (Kostrikis et al., 1998, Science 279:1228-1229).

[0026] Two molecular beacon oligonucleotides are "spectrally distinct" if they can be differentially detected using spectrophotometric or spectrofluorimetric methods. Examples of characteristics that can be used to differentiate spectrally distinct oligonucleotides include absorption or excitation wavelength, emission wavelength, and fluorescent lifetime.

[0027] An "instructional material" is a publication, a recording, a diagram, or any other medium of expression which can be used to communicate how to use a kit described herein, numerical values for weighting the significance of various polymorphisms that are detectable using the kit, or both. The instructional material of the kit of the invention can, for example, be affixed to a container which contains a kit of the invention or be shipped together with a container which contains the kit. Alternatively, the instructional material can be shipped separately from the container with the intention that the instructional material and the kit be used cooperatively by the recipient.

[0028] The "stringency" with which two polynucleotides anneal means the relative likelihood that the polynucleotides will anneal in a solution as the conditions of the solution become less favorable for annealing. Examples of stringent conditions are known in the art and

can be found in available references (e.g., Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 1989, 6.3.1-6.3.6). Aqueous and non-aqueous annealing methods are described in that reference and either can be used. In general, a first pair of polynucleotides anneal with higher stringency than a second pair if the first pair is more likely to anneal (or remain
5 annealed) as one or more of the salt concentration, temperature, and detergent concentration are increased.

[0029] With respect to a disorder, a "correlation factor" for a disorder-associated polymorphism is the fractions of humans who are heterozygous or homozygous for the polymorphism who exhibit the disorder. The correlation factor can, alternatively, be based
10 solely on those who are heterozygous, solely on those who are homozygous, or on those who are either heterozygous or homozygous.

[0030] A "non-extendable" nucleotide residue is a nucleotide residue that is capable of being added to a polynucleotide by a polymerase (i.e., by extension of the polynucleotide in association with a complement thereof, catalyzed by the polymerase) and that, upon addition to
15 the polynucleotide, renders the polynucleotide incapable of being further extended by the polymerase.

Description

[0031] The invention relates to kits and methods for assessing the relative susceptibility of
20 a human to oxidative damage by assessing occurrence in the human's genome of genetic polymorphisms that are associated with disorders.

[0032] It has been discovered that the degree to which a human is susceptible to oxidative damage can be assessed by determining which polymorphic forms of certain genes are present in the human's genome. The genes which are assessed are genes that are associated with
25 oxidative stress, including both genes which provide protection against oxidative damage and genes which exacerbate oxidative damage.

[0033] Among the types of genes which protect the body against oxidative stress are genes which encode an enzyme that catalyzes conversion of a toxic oxygen species to a less toxic oxygen species, genes that encode a protein that directly provides protection against oxidative

damage, and genes which encode a protein that indirectly provides protection against oxidative damage.

[0034] Among enzymes that catalyze conversion of a toxic oxygen species to a less toxic oxygen species, four are of particular relevance, namely mitochondrial manganese superoxide

5 dismutase (MnSOD), cytoplasmic copper/zinc superoxide dismutase (CZSOD), catalase (CAT), and glutathione peroxidase (GP). Polymorphisms that occur in these genes are known to be associated with various disorders (see, e.g., Kimura et al., 2000, Am. J. Ophthalmol.

130:769-773). Occurrence of disorder-associated polymorphisms in at least one (and preferably two, three, or all) of these four genes should be assessed in the methods described

10 herein, given the importance of these genes. Similarly, the kits described herein preferably include reagents for detecting disorder-associated polymorphisms in at least one (and preferably two, three, or all) of these four genes. In addition, the significance of occurrence of disorder-associated polymorphisms in these genes can be applied by assigning a greater weighting factor to disorder-associated polymorphisms of these genes than to disorder-

15 associated polymorphisms in other genes associated with oxidative stress.

[0035] It was not previously appreciated that detection in a human's genome of two or more disorder-associated polymorphisms in genes associated with oxidative stress is indicative that the human globally exhibits enhanced susceptibility to oxidative damage. Previous studies are believed to have recognized only association between a polymorphism in one of these genes

20 and a particular disorder (e.g., exudative macular degeneration in the Kimura reference). The inventors believe that they are the first to describe methods and kits for assessing a human's global (i.e., not limited to a particular tissue, cell type, or organ) susceptibility to oxidative damage.

[0036] In addition to the MnSOD, CZSOD, CAT, and GP genes mentioned above, other

25 genes encode proteins which provide direct or indirect protection against oxidative damage, for example by converting toxic species of oxygen to less toxic species, by eliminating precursors of toxic forms of oxygen, or by repairing oxidative damage. Examples of these genes include those which encode glutathione S-transferase, glutathione reductase, thioredoxin reductase, paraoxonase, NAD(P)H:quinone oxidoreductases 1 and 2, 8-oxo-7,8-dihydrodeoxyguanosine

triphosphatase, and epoxide hydrolase. Detection in a human genome of disorder-associated polymorphisms in one or more of these genes indicates that the human exhibits enhanced susceptibility to oxidative damage. The methods and kits described herein can use this indication to assess the susceptibility of a human to oxidative stress.

5 [0037] Among the genes which exacerbate oxidative damage are genes which encode a protein that induces production of a toxic oxygen species, either directly (e.g., by catalyzing a reaction in which a toxic species of oxygen is a direct or side product) or indirectly (e.g., by enhancing flux through a metabolic pathway that leads to production of a toxic species of oxygen). Examples of proteins that directly or indirectly induce production of toxic oxygen
10 species include myeloperoxidase, tumor necrosis factor alpha, NADH/NADPH oxidase p22 phox protein, nitric oxide synthase xanthine oxidase, and cytochrome P450. Detection in a human genome of disorder-associated polymorphisms in one or more genes encoding one of these proteins indicates that the human exhibits enhanced susceptibility to oxidative damage.

[0038] The methods described herein can also be used to assess susceptibility to oxidative
15 damage by determining the presence in a human's genome of polymorphic forms of genes that are associated with oxidative damage, regardless of whether the mechanism by which the gene affects oxidative stress is understood. By way of example, apolipoprotein E is a multi-functional molecule that is able to affect oxidative stress. The ApoE4 phenotype, for example, is known to be associated with enhanced hydroxyl radical levels in patients afflicted with
20 Alzheimer's disease, and ApoE expression is known to exacerbate oxidative stress. Further by way of example, enhancement of oxidative stress is known to be associated with each of elevated homocysteine level, depressed serum bilirubin level, depressed acid phosphatase activity, depressed protein phosphotyrosine phosphatase activity, and depressed epinephrine oxidase activity. Thus, occurrence in the genome of polymorphisms in genes which encode
25 proteins that affect these levels and activities can be determined, and their occurrence can be used to estimate susceptibility of the human to oxidative stress. Examples of genes for which polymorphisms can be associated with altered susceptibility to oxidative damage include UDP-glucuronosyltransferase 1A1 (i.e., the UGT1A1 gene), genes encoding acid phosphatase, protein phosphotyrosine phosphatase, epinephrine oxidase, ApoE4, cystathionine beta-

synthase, cystathionine gamma-lyase, N₅-methyl THF:homocysteine methyltransferase, and S-adenosylmethionine methyltransferase.

[0039] Heat shock proteins are also known to provide at least indirect protection of cells from oxidative damage, and occurrence of a heat shock protein gene polymorphism can be used as informative markers of susceptibility to oxidative damage when the polymorphism is known to be a disorder-associated polymorphism.

[0040] Examples of the polymorphisms in the foregoing genes which can be informative for susceptibility to oxidative damage include the following:

- a polymorphism manifested as a change from an alanine residue to a valine residue at amino acid residue 9 (i.e., in the signal sequence) of MnSOD;
- a polymorphism manifested as a change from an isoleucine residue to a thymine residue at amino acid residue 58 of MnSOD;
- a polymorphism manifested as a change from a valine residue to a glutamic acid residue at amino acid residue 7 of CZSOD;
- a polymorphism manifested as a change from a cysteine residue to a phenylalanine residue at amino acid residue 6 of CZSOD;
- a polymorphism manifested as a change from a cytosine residue to a thymine residue at nucleotide residue -262 (i.e., in the promoter region) of the catalase gene;
- a polymorphism in the hGPX1 gene manifested as a change from a proline residue to a leucine residue at amino acid residue 198 of glutathione peroxidase;
- a polymorphism in the GSTP1 gene manifested as a change from a valine residue to an isoleucine residue at amino acid residue 105 of glutathione peroxidase;
- a polymorphism manifested as a change from a thymine residue to a cytosine residue at nucleotide residue -107 (i.e., in the promoter region) of the gene which encodes paraoxonase;
- a polymorphism manifested as a change from a cytosine residue to a thymine residue at nucleotide residue 242 (i.e., in the coding region) of the gene encoding NAD(P)H:quinone oxidoreductase;

- a polymorphism manifested as a change from a thymine residue to a cytosine residue at nucleotide residue 113 in exon 3 of the gene which encodes epoxide hydrolase (i.e., effecting change of from a tyrosine residue to a histidine residue in epoxide hydrolase);
- a polymorphism manifested as a change from a guanine residue to an adenine residue at nucleotide residue -463 (i.e., in the promoter region) of the gene which encodes myeloperoxidase;
- a polymorphism manifested as a change to an adenine residue at nucleotide residue -238 (i.e., in the promoter region) of the gene which encodes tumor necrosis factor alpha (i.e., the TNF promoter variant designated TNF2);
- a polymorphism manifested as a change to an adenine residue at nucleotide residue -308 (i.e., in the promoter region) of the gene which encodes tumor necrosis factor alpha (i.e., the TNF promoter variant designated TNF3);
- a polymorphism manifested as a change from a cytosine residue to a thymine residue at nucleotide residue 242 (i.e., in the coding region) of the phox gene encoding the NADH/NADPH oxidase p22 subunit;
- a polymorphism manifested as a 27 base pair repeat in intron 4 (i.e., between nucleotide residues 5130 and 5511) of the gene encoding nitric oxide synthase;
- a polymorphism manifested as a change from an adenine residue to a guanine residue at nucleotide residue -290 (i.e., in the 5'-flanking region) of the gene encoding cytochrome P450 (i.e., the polymorphism designated the CYP3A4 cytochrome P450 variant);
- the polymorphism designated the ApoE4 allele of the ApoE gene; and
- a polymorphism manifested as a change from a cytosine residue to a thymine residue at nucleotide residue 699 (i.e., in the coding region) of the gene encoding cystathionine beta-synthase.

Methods of Assessing Susceptibility to Oxidative Damage

[0041] The invention includes a method of assessing the relative susceptibility of a human to oxidative damage. This susceptibility can be calculated relative to a hypothetical human whose genome does not contain a single disorder-associated polymorphism in a gene associated

with oxidative stress. Alternatively, susceptibility can be calculated relative to another human who may have one or more different disorder-associated polymorphism than the human being assessed. In practice, the basis upon which raw susceptibility scores are calculated is immaterial, so long as the same basis is used for all humans whose scores are to be compared
5 (i.e., so that the scores are relatable to one another).

[0042] The relative susceptibility of a human to oxidative damage permits assessment of risks and benefits of a variety of compositions, conditions, and interventions. In one embodiment, the susceptibility of a human to oxidative damage can be used to determine whether the human would benefit by supplementing nutritional intake with a composition that
10 contains one or more anti-oxidants. Furthermore, the relative susceptibility of the human to oxidative damage can indicate an appropriate dose of such an anti-oxidant-containing composition. In another embodiment, suitability of a condition or intervention for a human (e.g., administration to the human of hyperbaric oxygen or a pharmaceutical agent known to induce generation of toxic species of oxygen) can be determined by assessing the human's
15 susceptibility to oxidative damage.

[0043] Susceptibility of a human to oxidative damage is assessed by assessing occurrence in the human's genome of disorder-associated polymorphisms in a plurality of genes associated with oxidative stress (e.g., 3, 4, 6, 8, 10, 15, 20, or 30 or more genes). Occurrence of a disorder-associated polymorphism in one of these genes is an indication that the human has a
20 greater susceptibility to oxidative damage than a human in whose genome the polymorphism does not occur. Of course, occurrence of two or more such polymorphisms in the human's genome indicates that the human exhibits even greater susceptibility to oxidative damages.

[0044] Occurrence of every disorder-associated polymorphisms in a gene related to oxidative stress is not necessarily equally indicative of susceptibility to oxidative stress. In
25 order to account for differences in the significance of various disorder-associated polymorphisms, a weighting factor can be assigned to each polymorphism detected in the methods and kits described herein. As indicated above, four genes (MnSOD, CZSOD, CAT, and GP) are known to have very significant roles in oxidative stress in humans. All else being equal, disorder-associated polymorphisms that occur in one of these four genes are more

significant than polymorphisms that occur in genes having less significant roles in oxidative stress. Thus, a greater weighting factor can be assigned to these polymorphisms than to others. By way of example, the weighting factor assigned to these four polymorphisms can be 1 to 10 times greater than the weighting factor assigned to disorder-associated polymorphisms (having
5 equal correlation with the corresponding disorder, as discussed below) in other genes. Preferably, the weighting factor assigned to polymorphisms in the MnSOD, CZSOD, CAT, and GP genes is twice that assigned to disorder-associated polymorphisms in other genes.

[0045] Another factor which can influence the significance that is assigned to occurrence of a disorder-associated polymorphism in a human's genome is the degree to which the

10 polymorphism is correlated with the corresponding disorder. Some disorders are highly correlated with occurrence of a genetic polymorphism, and other disorders exhibit lower correlation with a polymorphism. When a polymorphism is reported to be associated with a disorder (i.e., with a disease or pathological condition), a degree of correlation between the polymorphism and the disorder is often reported. One useful way of calculating a factor that
15 describes correlation between a polymorphism and a disorder is to calculate an odds ratio that describes the likelihood that an individual in whose genome the disorder-associate polymorphism occurs will exhibit or develop the disorder. Because the kits and methods described herein can be used to detect whether the human is homozygous for the disease-associated polymorphism, odds ratios calculated for homozygous individuals can also be used,
20 if they are available. Odds ratios can be calculated as described in the art.

[0046] For a disorder-associated polymorphism, the odds ratio can be calculated as follows. First, the odds of being afflicted with the disorder are calculated for a first population in whom the polymorphism occurs by dividing the number of afflicted individuals in the first population by the total number of individuals in the first population. Second, the odds of being afflicted
25 with the disorder are calculated for a first population in whom the polymorphism does not occur by dividing the number of afflicted individuals in the second population by the total number of individuals in the second population. Third, the odds ratio is calculated by dividing the odds for the first population by the odds for the second population. If the odds ratio is greater than one, then this is an indication that occurrence of the polymorphism is associated with

occurrence of the disorder. Furthermore, the magnitude of the odds ratio is an indication of the significance of the association.

[0047] An overall oxidative stress susceptibility score for a human can be determined as follows. A significance score can be assigned to each disorder-associated polymorphism that is detected in the human's genome using a method or kit described herein. The significance score is a constant (e.g., 1.00), and is multiplied by any significance factor (e.g., 1-10, preferably 2, for the MnSOD, CZSOD, CAT, and GP genes) and by any correlation factor that is available. If information is available which describes the correlation between homozygosity for the polymorphism and the corresponding disorder, then that correlation factor should be used in place of the correlation factor for mere occurrence of the polymorphism, at least if the method or kit is used to rule out occurrence in the subject's genome of corresponding non-disorder-associated polymorphisms. If significance and correlation factors are not available, then values of 1.00 should be assigned to each. An overall score is determined by summing the significance score for each disorder-associated polymorphism that is detected using the method or kit. This overall oxidative stress susceptibility score can be compared with the values obtained from other subjects, or it can be compared with the value (i.e., zero) which would be expected to occur in a human whose genome does not include any disorder-associated polymorphism in a gene associated with oxidative stress.

[0048] By way of example, the Kimura reference describes two corresponding polymorphisms that occur in the MnSOD gene (i.e., occurrence of either C or T at a particular position in the MnSOD gene). Individuals in whose genome the disorder-associated polymorphism occur exhibit an odds ratio of 1.43 for the disorder (a form of macular degeneration), and individuals who are homozygous for the same polymorphism exhibit an odds ratio of 10.14. Thus, when the MnSOD gene is one of the genes assessed in the methods and kits described herein, a weighting factor of 1.43 can be applied to occurrence of this disorder-associated polymorphism in the subject's genome, and a weighting factor of 10.14 can be applied if the method or kit is used to determine that no other corresponding polymorphism occurs in the subject's genome. As indicated herein, an additional factor can be combined with this factor to represent the significance of the MnSOD gene in oxidative stress. Thus, if this

latter factor is selected to be 2, then occurrence of the disorder-associated polymorphism described in Kimura can be assigned a significance of 2.86, and exclusive occurrence of that polymorphism (i.e., homozygosity) can be assigned a significance of 20.28.

[0049] The method used to assess occurrence of any particular disorder-associated

polymorphism (or non-disorder-associated polymorphism) is not critical. Numerous methods of detecting occurrence of a polymorphism are known in the art, and substantially any of those methods can be used in the kits and methods described herein. Naturally, the reagents included in the kit will vary depending on the method to be used to detect the polymorphisms. Examples of some suitable polymorphism detection methods are provided below.

[0050] In one embodiment, a pair of oligonucleotide primers are used to amplify a portion of the gene that includes a polymorphic region. Detection of one or more of the polymorphisms that occur at the polymorphic region can be achieved by contacting the amplified portion with an oligonucleotide having a sequence that it will anneal under stringent conditions with the amplified portion only if one polymorphism occurs at the portion, but will not anneal with the amplified portion if another polymorphism occurs at that portion. Various acceptable stringent conditions are known in the art, and can be modified by the skilled artisan as appropriate to any particular amplified portion/oligonucleotide pair. An example of stringent conditions is hybridization in 6× sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2× SSC, 0.1% (w/v) SDS at 50°C.

[0051] In an alternative embodiment, one or more molecular beacon oligonucleotides are used to detect polymorphisms (disorder-associated, non-disorder-associated, or both) in a sample that contains a copy of the subject's genome, a fraction of the subject's genome, or amplification products generated from the subject's genome (e.g., amplified portions of oxidative stress-associated genes in which portions polymorphisms are known to occur).

[0052] Molecular beacon probes are single-stranded oligonucleotides having a fluorescent label (e.g. rhodamine, FAM, TET, VIC, JOE, or HEX) attached to the 5'-end thereof and a fluorescence quencher (e.g. TAMRA or DABCYL) attached to the 3'-end thereof (or vice versa), as described (Kostrikis et al., 1998, Science 279:1228-1229). The sequence of each molecular beacon probe is selected to include two complementary hairpin regions, whereby the

probe can self-anneal to form a hairpin structure. The 5'- and 3'- ends are brought into close association when the hairpin structure forms. The probe also comprises a targeting portion which is selected to be complementary to a target sequence (e.g. a single polymorphism of an oxidative-stress-associated gene). The targeting portion and at least one of the hairpin regions are located in close proximity to one another, meaning that the targeting portion either overlaps the hairpin region or flanks it, having no more than about 5 nucleotide residues therebetween.

[0053] If the hairpin regions of the molecular beacon probe anneal with one another, then the probe does not fluoresce, because the hairpin structure forms and the fluorescence quencher attached to one end of the probe quenches fluorescence of the label attached to the other end of the probe. If the targeting portion of the probe anneals with a region of a nucleic acid having the target sequence, then formation of the hairpin structure is inhibited, the fluorescence quencher is not brought into association with the fluorescent label, and the probe fluoresces. Multiple molecular beacon probes can be used in a single reaction mixture, and fluorescence associated with the probes can be differentiated if the molecular beacon probes are spectrally distinct.

[0054] Thus, in this embodiment, one or more molecular beacon probes are used, each having targeting portion which is complementary to a target region (e.g. 20 to 40 nucleotide residues, more preferably 20 to 30 residues) of one polymorphism of an oxidative stress-associated gene (e.g., one of the genes disclosed herein). If the polymorphism to be detected is a single nucleotide polymorphism (SNP), then the target region includes, and preferably is approximately centered around, the nucleotide residue at which the polymorphism occurs. More preferably, two such probes are used, one having a targeting region completely complementary to the target region of one polymorphism of the gene (e.g., one of two polymorphisms of an SNP), and the other having a targeting region completely complementary to the target region of a corresponding polymorphism of the gene (e.g., the other polymorphism of the SNP).

[0055] In yet another embodiment of how polymorphisms in an oxidative damage-associated gene can be assessed, oligonucleotide primers which are complementary to a region adjacent a characteristic residue of the polymorphism are extended using a polymerase enzyme,

and the identity of the nucleotide residue that is added to the primer in the position complementary to the characteristic residue is determined. The primer can be extended in the presence of non-extendable nucleotide residues in order to ensure that a limited number of (or only one) nucleotide residues are incorporated into the primer. Methods of this type are known in the art (e.g., the SNP-IT® technology of Orchid Biocomputer, Inc.) and are described, for example in U.S. Patents numbers 6,013,431 and 6,004,744.

Kits for Assessing Oxidative Stress

[0056] The invention includes a kit for assessing the relative susceptibility of a human to oxidative stress. The kit contains reagents for performing one or more of the methods described herein. The reagents used in certain embodiments of the methods described herein are indicated above. Reagents useful for performing those methods using a variety of alternative sample preparation and polymorphism detection methods or chemistries are apparent to the skilled artisan.

[0057] Kits for detecting polymorphisms in individual genes are known in the art, and the kit of the invention can have similar components. However, a critical feature of the kit is that it includes reagents that permit its user to detect disorder-associated polymorphisms in at least three genes associated with oxidative stress. Preferably the kit includes reagents that permit detection of disorder-associated polymorphisms in at least 4, 6, 8, 10, 15, 20, or 30 or more such genes.

[0058] In one embodiment, the kit includes a plurality of oligonucleotides which anneal under stringent conditions with a disorder-associated polymorphism of one of the genes, but not with a non-disorder associated-polymorphism. Each of the oligonucleotides is preferably attached to a surface in order to facilitate handling of the oligonucleotide. The oligonucleotides can be linked with a plurality of surfaces (e.g., oligonucleotides for a particular polymorphism being attached to a particle discrete from a particle to which oligonucleotides for another polymorphism are attached), or they can be attached to discrete regions of a single surface (e.g., as in the GENECHIP™ device of Affymetrix, Inc.). Annealing between individual oligonucleotides and the polymorphism corresponding thereto can be detected using standard

methods. The kit can also comprise oligonucleotides that are useful as molecular beacon probes or as extendable primers.

[0059] In one embodiment, the kit further comprises a DNA collection kit or apparatus, such as that described in co-pending U.S. patent application number 09/302,623 (allowed).

5 Advantageously, DNA collected using the kit or apparatus can be stored or archived, and subjected to additional testing as previously unknown polymorphisms are discovered in genes associated with oxidative stress, or as the significance of previously unappreciated polymorphisms is realized.

[0060] It will be appreciated by those skilled in the art that changes can made to the
10 embodiments described above without departing from the broad inventive concept thereof.

[0061] This invention is not limited to the particular embodiments disclosed, and includes modifications within the spirit and scope of the present invention as defined by the appended claims.